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基于斑马鱼全生命周期毒性测试的研究进展

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摘要: 作为一种模式生物,斑马鱼具有很多优点,包括体积小、成本低、适应性广、繁殖周期短、胚胎透明且产卵量高等,因而被广泛应用于生态毒理学等领域。斑马鱼的生命阶段主要包括胚胎、仔鱼和成鱼 3 个阶段。近年来,斑马鱼全生命周期的毒理学研究所占比重不断上升并呈稳定增长的趋势。本文对斑马鱼 3 个生命阶段及整个生命周期的毒性试验展开综述,介绍了斑马鱼全生命周期实验在毒理学中的研究进展,总结了各生命阶段国内外的标准规范和资源库信息、毒理学终点及转基因斑马鱼的研究进展,归纳了多阶段毒性评价和整个生命周期毒性评价的应用。最后,对全生命周期和基于斑马鱼毒性测试的应用做出展望,对未来开展的相关研究提出建议。

关键词: 斑马鱼;生命周期;毒性测试
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Research Progress of Life-cycle Toxicity Test of Zebrafish

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Abstract: As a model organism, zebrafish (*Danio rerio*) is widely used in ecotoxicology and other fields as its small size, low cost, wide adaptability, short breeding cycle, high fertility and embryonic transparency. The life stage of zebrafish mainly includes embryo, larvae and adult. The proportion of toxicology research of zebrafish life cycle continued to rise and showed a steady growth trend in recent years. In present study, the toxicity tests of ze-

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brafish in three life stages and the whole life cycle was introduced and reviewed. Secondly, toxicity test guideline and standard of zebrafish in various life stages, resource library information, endpoint and transgenic zebrafish were also summarized. Thirdly, the application of multi-stage and whole life cycle toxicity assessment were generalized. Finally, the prospects of the application of zebrafish toxicity testing and the whole life cycle were discussed and suggested in future research.

Keywords: zebrafish; life-cycle; toxicity test

斑马鱼(zebrafish, *Danio rerio*)是一种原产于印度和南亚河流的小型热带鱼[1],自1994年起作为一种全新的脊椎动物模型被学术界接受[2-3],它的器官、细胞类型、心血管系统、消化系统与哺乳动物相似[4-5]。另外,斑马鱼与人类的遗传和生理同源性显著[6],大约70%的人类基因至少有一个明显的斑马鱼直系同源物[7],神经系统组织和代谢与人类类似[8-9],生物学特性接近于人类生长全过程[10],它还有易于获得、价格低廉、容易饲养、符合"3R"原则、测试周期短并且在适当的光周期条件下能产出大量粘附性低、可浸泡、透明的鱼卵等优点[11-13],在生态毒理学和发育遗传学等领域中得到了广泛的研究[14],相对于小鼠、果蝇和线虫等模式生物,近年来斑马鱼的应用地位显著[15]。

斑马鱼的生命阶段主要包括胚胎(卵裂期、囊胚期、原肠胚期、分裂期、成形期和孵化期)、仔鱼和成鱼3个阶段,72 hpf(hour post fertilization, hpf)进入早期仔鱼阶段且主要器官发育完成,96 hpf时大多数器官发育完成,120 hpf时仔鱼发育完成[16-17]。生命周期试验主要分为2类:整个生命周期试验(full life cycle test)和部分生命周期试验(partial life cycle test)[18],毒性主要包括一般毒性(急性毒性、亚慢性毒性、慢性毒性、蓄积毒性和局部毒性等)和特殊毒性(致癌作用、致突变作用、生殖和发育毒性等)[19]。利用PubMed数据库,以zebrafish embryo, zebrafish larvae, zebrafish adult和zebrafish life cycle为限定条件,以toxicology为限定区域统计发现,近10年来,斑马鱼生命周期的毒理学研究所占比重不断上升并呈稳定增长的趋势(图1)。本文综述了斑马鱼全生命周期毒性的研究进展,为深入研究毒性物质对鱼类以及其他高等脊椎动物生长发育的影响提供一定的参考依据。

1 斑马鱼各生命阶段在毒理学中的研究进展(Research progress on partial life cycle test of zebrafish in toxicology)

1.1 斑马鱼胚胎在毒理学中的研究进展

1.1.1 急性毒性及发育毒性的研究进展

急性毒性是指机体(人或实验动物)一次或短期内(一般指24～96 h)多次接触外源化合物之后所引起的中毒效应,这种毒性效应可以反映在不同的组

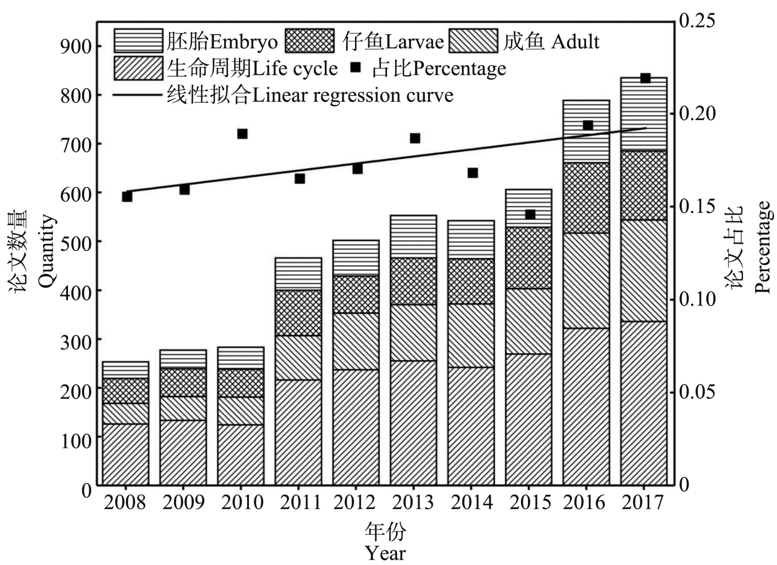

图1 斑马鱼生命周期相关的论文及占比

Fig. 1 Statistical analysis of scientific papers related to zebrafish life cycle

织水平上,并伴随着不同的症状,甚至死亡[19]。20 世纪 70 年代,Laale[20]对斑马鱼胚胎在生态毒理学领域的应用进行了报道,后来 Lange 等[21]使用斑马鱼及其胚胎和 RTG-2 细胞进行毒性试验,结果证明,胚胎测试对毒性反应最敏感,并且认为胚胎毒性测试是替代鱼类急性毒性测试的最佳方法。

2002 年 Nagel[22]进一步证明斑马鱼胚胎可以替代鱼类 96 h 的急性毒性试验,同年,德国率先使用斑马鱼胚胎 48 h 毒性试验(fish embryo toxicity, FET)代替鱼类急性毒性试验,自 2005 年以来,FET 已成为德国全废水毒性试验(whole effluent test, WET)的重要组成部分[23]。在进行斑马鱼胚胎急性毒性测试的同时,也可以筛选发育不正常的胚胎作为致畸作用指标,相关研究人员总结斑马鱼胚胎形态学、亚致死等毒性终点(表 1),如心跳变化、运动行为和孵化率等,这些终点丰富了毒性内容,且表明了化学物质可能存在的发育毒性和慢性毒性效应[24]。

1.1.2　内分泌干扰毒性的研究进展

内分泌干扰毒性的主要原因在于天然激素水平的失衡,而引起激素水平失衡的物质大多数来源于人类的生产和生活过程中产生和排放的污染物,由于这类物质具有与生物体内激素相同的活性,会影响生物内分泌系统的正常工作,因此称之为“环境内分泌干扰物(environmental endocrine disrupters, EEDs)”[25−26]。目前,大多数报道以特定的终点如性别分化或诱导卵黄蛋白原(vitellogenin, VTG)来研究对雌激素、雄激素和甲状腺受体起作用的 EEDs 的影响[27]。斑马鱼胚胎模型在内分泌干扰毒性研究中的应用是通过观察 EEDs 暴露对激素应答基因的影响进行检测筛选,VTG 作为敏感型的分子生物标记物,已被广泛应用于斑马鱼胚胎模型中[28]。然而,在

表 1　斑马鱼胚胎急性毒性和致畸性的终点[22]

Table 1　Lethal and sublethal endpoints for evaluating the acute toxicity and teratogenicity of chemicals on zebrafish embryos [22]

毒性终点 Toxicological endpoint	指标 Index	暴露时长/h Exposure time/h			
		8	24	48	120
致死终点* Lethal*	卵凝结 Coagulation	●	●	●	
	尾部未脱离 Tail not detached		●	●	
	体节未形成 No somites		●	●	
	无心跳 No heart-beat			●	
亚致死/发育毒性终点 Sublethal/Development	原肠胚形成 Completion of gastrula	●			
	体节形成 Formation of smites		●		
	眼点形成 Development of eyes		●	●	
	自主运动 Spontaneous movement		●	●	
	心跳/血液循环 Heart beat/blood circulation			●	
	色素沉着 Pigmentation			●	
	水肿形成 Oedema			●	
致畸终点 Teratogenic	头部畸形 Malformation of head		●	●	
	球囊/耳石畸形 Malformation of sacculi/otoliths		●	●	
	尾部畸形 Malformation of tail		●	●	
	心脏畸形 Malformation of heart		●	●	
	脊柱弯曲 Modified structure of the corda		●	●	
	卵黄畸形 Deformity of yolk		●	●	
	生长迟缓 Growth-retardation		●	●	
	尾部长度** Length of tail**				●

注:* 在致畸毒性试验中,只有卵凝结判定为死亡;** 暴露 48 h 后将胚胎转移到没有试验化合物的水中;120 hpf 仔鱼变直,可以精确地测量尾长。

Note: * Within the teratogenicity test for a better comparison with mammalian data only the endpoint "coagulated" is used as a lethal effect. ** In this case the eggs are transfered into water without the test compound after 48 h. After natural hatching the larvae is straightened and the tail length can be determined.

斑马鱼胚胎模型中尚未有雄激素和糖皮质激素的报道,对于这些终点的检测原则上可以通过转基因报告菌株分析其应答基因,但是现有的菌株,如 pERE tata-Luc,只被成功地运用到仔鱼或成鱼阶段[29]。对内分泌干扰毒性的分析,特别是对不直接干扰受体调控基因(如性别分化),需要在仔鱼敏感的发展阶段进行。因此,斑马鱼胚胎可以作为简单的筛选工具,但在研究更复杂的激素调节过程中的应用尚待开发。

对斑马鱼胚胎的毒理研究还涉及神经毒性、遗传毒性和基因技术等领域[30]。MacRae 和 Peterson[31]还报道了斑马鱼及其胚胎在药物开发中的应用,并提出斑马鱼模型与传统药物开发方法的结合有助于平衡药物毒性和疗效。斑马鱼胚胎在毒理学研究中的前景主要是:(1)确定更合适的分子标记物作为毒性测试指标(如内分泌干扰毒性);(2)建立短期毒性测试(如急性毒性)和长期毒性测试(如慢性毒性和发育毒性)之间的关系;(3)斑马鱼胚胎模型的开发及应用可以为环境管理决策服务;(4)斑马鱼及其胚胎在医学领域的研究可以扩大其在毒理学的应用。

1.2 斑马鱼仔鱼及成鱼在毒理学中的研究进展

一般在完成孵化后 48 ~72 h 的斑马鱼可以自由运动并且能够自主取食,标志着正式进入了仔鱼期,斑马鱼仔鱼发育要持续数周,因此,目前针对仔鱼的毒性试验除短期暴露外,还有长期暴露试验。由于斑马鱼仔鱼具备自主游动能力,因此,一些工作以仔鱼行为作为评价指标,进行毒性试验。斑马鱼成鱼四月龄进入性成熟期,一般 5 ~6 月龄的斑马鱼开始被用于成鱼毒性试验中。由于斑马鱼成鱼体内的各种生理生化系统和内分泌系统已经发育完毕,所以能用于毒性评价的指标非常丰富,成年斑马鱼相比于胚胎和仔鱼,最大的优势在于可以取出处理后斑马鱼体内的不同器官,来具体评价污染物对不同器官或组织的影响[32]。斑马鱼相对于其他模式生物应用于毒性检测而言,节省了时间和人力成本,具有快速、简便和高效的优势,降低了人和生态系统面临的毒性风险,是一种应用潜力更大的环境污染应急预警方法,被国际标准化组织(International Organization for Standardization, ISO)、经济合作与发展组织(Organization for Economic Co-Operation and Development, OECD)和欧盟等组织认定为标准毒性测试生物(表 2)。

野生型斑马鱼在毒理学中得到了广泛的应用,但这种方法仍有不足之处,如检测灵敏度度相对较低,实验过程及结果统计相对繁琐等。随着转基因技术的日益成熟,将具有特异性调控作用的基因导入斑马鱼体内,从而产生针对不同种类污染物可产生特异性表型的转基因斑马鱼,使针对不同污染物的分子生物标志物数据库不断扩展,为污染物环境监测与预警提供更多的数据支持[33],为环境样品中关键致毒物质筛查提供新的解决思路[34]。

目前,应用于环境监测的转基因斑马鱼主要有以下 5 种类型:芳香烃反应元件(aromatic hydrocarbon response element, AHRE)、亲电反应元件(electrophile response element, EPRE)、金属离子反应元件(metal response element, MRE)、雌激素反应元件(estrogen response element, ERE)和维甲酸和类维生素 X 反应元件(retinoic acid and retinoid X response elements, RAREs, RXREs)(表 3)。随着科学技术手段的不断进步,将来会有更多的转基因(反应元件)斑马鱼品系建立。各种带有不同反应元件的转基因斑马鱼胚胎可以像基因芯片一样排列在微孔板中,可以准确地检测出水环境中各种污染物的种类,并根据荧光强度来推算污染物的浓度[48-49]。Petersen 等[44]通过暴露转基因斑马鱼 tg(cyp19a1b-GFP)胚胎监测 5 种不同的环境雌激素,结果发现,所潜在的和弱的类雌激素都能够监测到。此外,Pawar 等[38]将转基因斑马鱼(pTol2-MT-Ia1-DsRed2)的胚胎暴露至 Cd^{2+}、Cu^{2+}、Hg^{2+}、Pb^{2+} 和 Zn^{2+} 溶液中,通过观察荧光强度确定毒性强弱。

2 斑马鱼多阶段毒性评价及整个生命周期的毒理学研究进展(Research progress on multi-stage and complete life cycle toxicity evaluation)

2.1 斑马鱼多阶段毒性评价及整个生命周期在毒性测试中的应用

随着对鱼类毒性试验了解的不断深入,人们逐渐发现传统的单一生命阶段毒性试验提供的数据较为片面,不能客观全面地反映有毒化合物对生物的毒性,大量研究已经发现,对于同一有毒污染物,不同生命阶段的鱼会表现出不同的敏感性和中毒反应,多阶段毒性评价能够避免由生命阶段不同而导致的敏感性差异[50-51]。通过整个生命周期毒性试验可以发现低剂量有毒化合物暴露的影响,并有助于建立长期化学品的安全浓度,提供可能的延迟毒性

表 2　利用斑马鱼生命周期开展毒性测试的标准

Table 2　Standard for zebrafish life-cycle toxicity test

生命周期 Life cycle	毒性类别 Toxicity category	标准制订主体 Setting department	标准名称 Standard name	标准编号 Standard coding	毒性终点/指标 Endpoint/index	采用时间 Date
胚胎 Embryo	急性毒性 Acute toxicity	OECD	鱼类胚胎和卵黄囊仔鱼阶段的短期毒性试验 Fish, short-term toxicity test on embryo and sac-fry stages	OECD 212	卵凝结,尾部未脱离,体节未形成,无心跳等 Coagulation, tail not detached, no somites, no heart-beat	1998
			鱼类胚胎急性毒性(FET)试验 Fish embryo acute toxicity (FET) test	OECD 236		2013
		ISO、欧盟及其成员国 ISO, European Union and its member states	水质 废水对斑马鱼卵的急性毒性的测定(*Danio rerio*) Water quality—Determination of the acute toxicity of wastewater to zebrafish eggs (*Danio rerio*)	ISO 15088 EN ISO 15088 BS EN ISO 15088 DIN EN ISO 15088		2007 2008 2008 2009
仔鱼 Larvae	急性毒性 Acute toxicity	OECD	鱼类早期生活阶段毒性试验 Fish, early-life stage toxicity test	OECD 210	LC_{50}	1992
	发育毒性 Developmental toxicity	OECD	鱼类幼体生长试验 Fish, juvenile growth test	OECD 215	EC_{50}	2000
	慢性毒性 Chronic toxicity	OECD	鱼类的生物富集:水和膳食暴露 Bioaccumulation in fish: Aqueous and dietary exposure	OECD 305	LC_{50}	2012
	内分泌干扰毒性 Endocrine disruption toxicity	OECD	鱼类性发育测试 Fish sexual development test	OECD 234	卵黄蛋白原(VTG)浓度,性别比 Vitellogenin concentration, sex ratio	2011
成鱼 Adult	急性毒性 Acute toxicity	OECD	鱼类延长毒性 14 d 试验 Fish, prolonged toxicity test: 14 day study	OECD 204	NOEC	1984
		OECD	鱼类急性毒性试验 Fish, acute toxicity test	OECD 203	LC_{50}	1992
		ISO	水质 物质对淡水鱼的急性毒性的测定 Water quality—Determination of the acute lethal toxicity of substances to a freshwater fish *Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)	ISO 7346-1	LC_{50}	1996
	内分泌干扰毒性 Endocrine disruption toxicity	OECD	21 d 鱼类测试 21 day fish assay	OECD 230	VTG 浓度 Vitellogenin concentration	2009
		OECD	鱼类生殖毒性短期试验方法 Fishshort term reproduction assay	OECD 229	VTG 浓度 Vitellogenin concentration	2012
		ISO	水质 鱼类的生化和生理测试第 3 部分:卵黄蛋白原的测定 Water quality—Biochemical and physiological measurements on fish—Part 3: Determination of vitellogenin	ISO 23893-3	VTG 浓度 Vitellogenin concentration	2013

注:LC_{50} 表示半数致死浓度,EC_{50} 表示半数效应浓度,NOEC 表示无可观察效应浓度。OECD 表示经济合作与发展组织,ISO 表示国际标准化组织。

Note: LC_{50} stands for median lethal concentration; EC_{50} stands for median effect concentration; NOEC stands for no observed effect concentration; OECD stands for Organization for Economic Co-Operation and Development; ISO stands for International Organization for Standardization.

表3 不同反应元件的转基因斑马鱼在环境监测中的应用

Table 3 Application of transgenic zebrafish with different response elements in environmental monitoring

反应元件 Response element	共有序列 Consensus sequence	活化剂 Activating agents	转录因子 Transcription factors	转基因斑马鱼系列 Transgenic zebrafish line	参考文献 Reference
AHRE	TWGCGTG	二噁英类化合物 Dioxin-like compounds	芳香烃受体,芳香烃受体核转位子蛋白异源二聚体 Aromatic hydrocarbon receptor, Ah receptor nuclear translocator protein heterodimer	Tg(cyp1a-12DRE:EGFP)	[35]
		多氯二苯并对呋喃(PeCDF)、多氯联苯(PCB126) Polychlorinated dibenzo-p-furan (PeCDF) and co-planar polychlorinated biphenyl (PCB126)		Tg(cyp1a:gfp)	[36]
		多环芳烃和2,3,7,8-四氯二苯并二噁英(TCDD) Polycyclic aromatic hydrocarbons and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)		Tg(cyp1a:mCherry)	[37]
MRE	TGCRCNCGG	Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Zn^{2+}	金属响应转录因子(MTF-1) Metal-responsive transcription factor-1 (MTF-1)	pTol2-MT-Ia1-DsRed2	[38]
		Zn, Cd		mt:egfp	[39]
		Cd, As		hsp27-gfp	[40]
ERE	GGTCANNNTGACC	17α-雌二醇(EE2)、双酚A(BPA)、金雀异黄素 Ethinylestradiol (EE2), bisphenol A (BPA), genistein	雌激素受体同源二聚体 Estrogen receptor homodimer	ERE-GFP-Casper	[41-42]
		17α-雌二醇(EE2)、17β-雌二醇(E2)、雌酮(E1)、双酚A(BPA)、4-叔辛基苯酚(OP) 17α-ethinylestradiol (EE2), 17β-estradiol (E2), estrone (E1), bisphenol A (BPA), 4-tert-octylphenol (OP)		5xERE:GFP	[43]
				tg(cyp19a1b-GFP)	[44]
EPRE	RTGACNNNGC	平面芳烃、强效亲电试剂 Planar aromatic hydrocarbons, potent electrophiles	NF-E2相关因子1、NF-E2相关因子2、小Maf NF-E2-related factor 1, NF-E2-related factor 2, small Maf	EPRE-Luc, mEPREmt1	[45]
RAREs	RGGTCA(N_{0-8})RGGTCA	维甲酸 Retinoids	维甲酸受体同源二聚体、类维生素X受体异源二聚体和同源二聚体 Retinoic acid receptor homodimers, heterodimers with retinoid X receptor, retinoid X receptor homodimers	RARE-tk, RARE-GT2	[46-47]
RXREs	GGGGTCAAAGGTCA GGGGTCATGGGGTCA				

注:AHRE表示芳香烃反应元件,MRE表示金属离子反应元件,ERE表示雌激素反应元件,EPRE表示亲电反应元件,RAREs表示维甲酸反应元件,RXREs表示类维生素X反应元件。共有序列中,N=A, T, G或C;R=A或G;W=A或T。

Note: AHRE stands for aromatic hydrocarbon response element; MRE stands for metal response element; EPRE stands for electrophile response element; RAREs stands for retinoic acid response element; RXREs stands for retinoid X response elements. Within each consensus sequence, N = A, T, G, or C; R = A or G; W = A or T.

的详细信息[52]。在毒理学评价中,单个生命阶段的毒理学试验结果较为片面,而整个生命周期的毒性试验时间跨度较大,对外界环境要求较高,实际操作起来难度较大。因此,许多毒性试验选择 3 个单独的斑马鱼生命阶段(胚胎、仔鱼和成鱼)[53]。斑马鱼不同的生命阶段有着不同的毒性测试标准体系,在多阶段毒性评价中可以参照对应的标准(表 3)进行试验。笔者查阅 OECD、ISO、美国环境保护局(United States Environmental Protcction Agency, US EPA)和日本环境省等标准资源库发现,目前 US EPA(Fish Life Cycle Toxicity, 1996)和日本环境省(the Medaka (*Oryzias Latipes*) Full Life-Cycle Test Guideline, 2002)制定了鱼类整个生命周期毒性试验。

在鱼类毒性检测的环境污染物中主要分为重金属、内分泌干扰物和有机物[54]。最早提出鱼类生命周期毒性试验的 Mount 和 Stephan[55]将黑头呆鱼的整个生命周期暴露于马拉硫磷和丁氧基乙醇酯中定量分析黑头呆鱼的生长繁殖所受的影响。随着斑马鱼模型的开发和应用,越来越多的研究针对这 3 类物质开展斑马鱼多阶段毒性评价和整个生命周期毒性试验。通过不同的评价方法,不同的暴露时长,以形态变化、基因表达、行为和生理学特征等指标来评价污染物的毒性(表 4)。

2.2　国内斑马鱼及其毒性试验标准的发展

斑马鱼在我国生物科学中的研究始于 1998 年,孟安明院士在清华大学建立国内第一家以斑马鱼为模式动物的发育生物学实验室[49]。2000 年后,斑马鱼研究团体飞速增长,截至 2017 年,中国斑马鱼学者在国际斑马鱼信息中心(the Zebrafish Information Network, ZFIN)网站注册的独立实验室已达 100 个[56]。国家斑马鱼资源中心隶属于中国科学院水生生物研究所,其斑马鱼资源保藏库现拥有 1 200 多个斑马鱼品系和 10 000 余份冻存精子,可提供斑马鱼品系等资源服务、转基因和基因敲除等技术服务、养殖和健康等咨询服务,以及技术培训和学术会议服务等[15]。此外,国际也有着相当数量且实力雄厚的斑马鱼生物信息学数据库(表 5)。通过 Web of Science™ Core Collection (v5.29)数据库以斑马鱼为主题同时限定区域分析(不含港澳台) 可知,2000—2001 年中国斑马鱼相关研究论文在世界中的占比仅为 1.6%,之后中国斑马鱼论文占比持续增长,到了 2010 年首次突破 10%,而到了 2017 年,更是达到 22.9%(图 2),另搜索中国知网数据库发现,以“斑马鱼”为主题的中文文献已有 4 010 篇(1990—2017 年)。

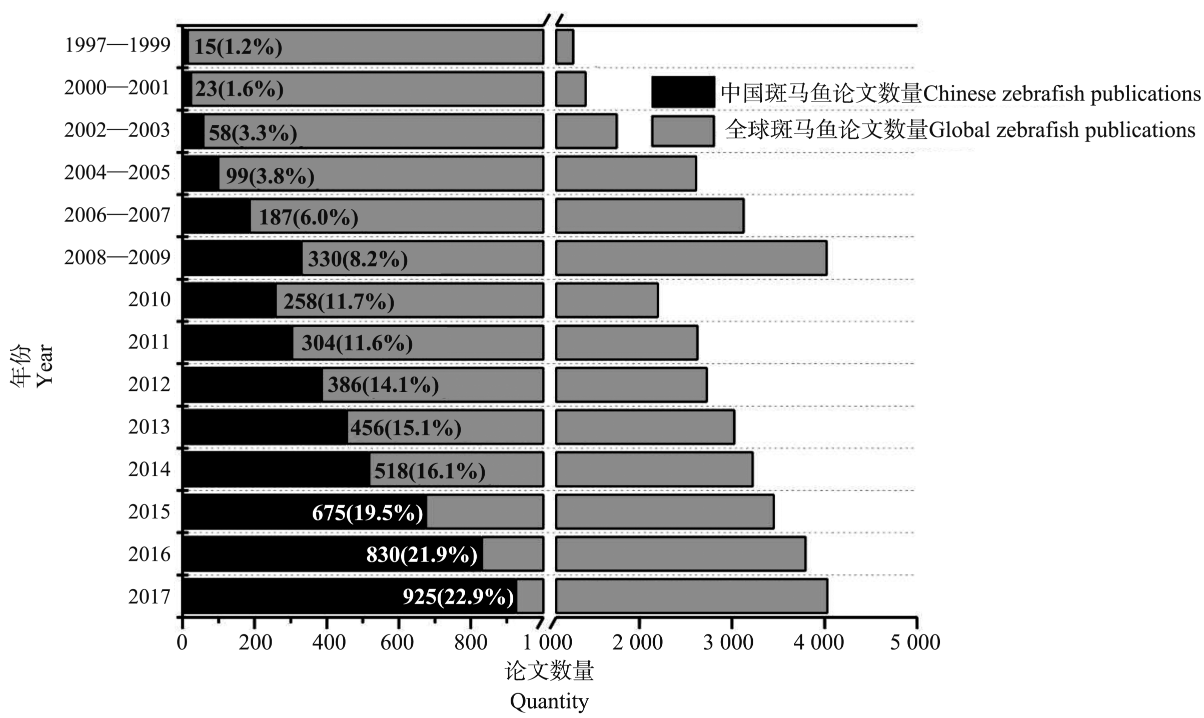

图 2　Web of Science™ Core Collection 数据库中 1997—2017 年中国斑马鱼论文数量及占比

Fig. 2　The proportion of Chinese zebrafish papers from 1997 to 2017 from Web of Science™ Core Collection datebase

表4 斑马鱼多阶段毒性评价和整个生命周期毒性评价的应用

Table 4 Application of multi-stage and complete life cycle toxicity evaluation

评价方法 Evaluation method	分类 Classification	污染物 Contaminants	暴露时长 Exposure time 胚胎 Embryo	 仔鱼 Larva	 成鱼 Adult	生物标记物/终点 Biomarker/endpoint	参考文献 References
多阶段毒性评价 Multi-stage toxicity evaluation	重金属 Heavy metal	Ni	10 d		96 h	外体形态、回避反应和运动行为 Morphology, avoidance response, locomotor behavior	[57-58]
		Pb, Hg, As, Cd	120 h			IL1β、TNF-α、IFNγ、Mx、Lyz、C3B和CXCL-Clc的mRNA表达水平 mRNA expression levels of IL1β, TNF-α, IFNγ, Mx, Lyz, C3B and CXCL-Clc	[59]
	有机物 Organic compounds	嘧菌酯 Azoxystrobin	96 h	96 h	96 h	孵化率、心率、生长抑制，心包水肿 Hatching rate, heart rate, growth inhibition, edema	[60]
		苯氧威 Phenoxyvir	96 h	96 h	96 h		[61]
		甲草胺、乙草胺、丙草胺 Alachlor, acetochlor, pretilachlor	96 h	96 h	96 h	96 h-LC_{50}	[62]
	内分泌干扰物质 Endocrine disrupting compounds	BPA	48 h	48 h	24 h	LC_{50}	[63]
		E2	3 d	7 d	7 d	VTG、Erα和Erβ的mRNA表达水平 mRNA expression levels of VTG, Erα and Erβ	[64]
整个生命周期毒性评价 Complete life cycle toxicity evaluation	重金属 Heavy metal	Cd	105 d			iNOS、TNF-α、NF-κB和IκBα基因的表达水平 mRNA expression levels of iNOS, TNF-α, NF-κB and IκBα	[64-65]
		甲基汞 MeHg	56 d			视觉-运动反应 Visual-motor response (VMR)	[66]
	有机物 Organic compounds	丁草胺 Butachlor	30 d			性腺轴相关基因表达 Gonadal axis-related gene expression	[67]
	内分泌干扰物质 Endocrine disrupting compounds	微囊藻毒素 Microcystin-LR	90 d			cyp19a1b、cyp19a1a、fshr和VTG基因表达 mRNA expression levels of cyp19a1b, cyp19a1a, fshr and VTG	[68]

表 5　斑马鱼生物信息学资源库

Table 5　Zebrafish bioinformatics resource library

名称 Name	机构 Institution	网址 Website	服务内容 Service
国家斑马鱼资源中心 China Zebrafish Resource Center, CZRC	中国科学院水生生物研究所 Institute of Hydrobiology, Chinese Academy of Sciences	http://zfish.cn	斑马鱼品系、养殖、技术培训和学术会议等 Zebrafish strains, breeding, technical training and academic conferences, etc.
斑马鱼模式物种综合数据库 the Zebrafish Information Network, ZFIN	美国俄勒冈大学 University of Oregon	http://zfin.org/	斑马鱼基因信息及相关资源的研究工作等 Research work on zebrafish genetic information and related resources, etc.
欧洲生物信息学研究所 the European Bioinformatics Institute, EBI	欧洲分子生物学实验室 European Molecular Biology Laboratory	https://www.ebi.ac.uk/	基因组自动注释平台 Genome automatic annotation platform
国家生物资源项目 National Bioresource Project-Zebrafish	日本国立遗传研究所 National Institute of Genetics, Japan	https://shigen.nig.ac.jp/zebra/index_en.html	建立收集、保存和提供斑马鱼品种的系统 Establish a system for collecting, preserving and providing zebrafish species
欧洲斑马鱼资源中心 the European Zebrafish Resource Center, EZRC	德国卡尔斯鲁厄理工学院 Karlsruhe Institute of Technology, Germany	http://www.ezrc.kit.edu/	斑马鱼遗传研究、高通量设施等 Zebrafish genetic research, high-throughput facilities, etc.
斑马鱼资源库 Zebrafish Resource Bank	韩国国家研究资源中心 Korea National Research Resource Center, South Korea	http://knrrb.knrrc.or.kr	斑马鱼及相关研究材料的收集、维护和储存,数据库建设等 Collection, maintenance and storage of zebrafish and related research materials, database construction, etc.

表6 国内斑马鱼相关标准制定进展

Table 6 Progress in the development of domestic zebrafish related standards

标准名称 Standard name	标准编号 Standard coding	标准状态 Standard status	实施日期 Implementation date
水质 物质对淡水鱼(斑马鱼)急性毒性测定方法 Water quality—Determination of the acute toxicity of substance to a freshwater fish (*Brachydanio rerio* Hamilton-Buchanan)	GB/T 13267—1991	现行 In operation	1992-08-01
危险化学品鱼类急性毒性分级试验方法 Test method of fish acute toxicity for dangerous chemical products	GB/T 21281—2007	现行 In operation	2008-07-01
工业废水的试验方法 鱼类急性毒性试验 Testing method for industrial wastewater—Fish acute toxicity	GB/T 21814—2008	现行 In operation	2008-09-01
化学品 鱼类幼体生长试验 Chemicals—Fish, juvenile growth test	GB/T 21806—2008	现行 In operation	2008-09-01
化学品 鱼类早期生活阶段毒性试验 Chemicals—Fish early-life stage toxicity test	GB/T 21854—2008	现行 In operation	2008-09-01
化学品 生物富集 流水式鱼类 Chemicals—Bioconcentration—Flow-through fish test	GB/T 21800—2008	现行 In operation	2008-09-01
化学品 生物富集 半静态式鱼类试验 Chemicals—Bioconcentration—Semi-static fish test	GB/T 21858—2008	现行 In operation	2008-09-01
化学品 鱼类胚胎和卵黄囊仔鱼阶段的短期毒性试验 Chemicals—Fish, short-term toxicity test on embryo and sac-fry stages	GB/T 21807—2008	现行 In operation	2008-09-01
化学品 鱼类延长毒性 14 d 试验 Chemicals—Fish prolonged toxicity: 14 day study test	GB/T 21808—2008	现行 In operation	2008-09-01
化学品 鱼类急性毒性试验 Chemicals—Fish acute toxicity test	GB/T 27861—2011	现行 In operation	2012-08-01
化学农药环境安全评价试验准则 第12部分：鱼类急性毒性试验 Test guideline on environmental safety assessment for chemical pesticides—Part 12: Fish acute toxicity test	GB/T 31270.12—2014	现行 In operation	2015-03-11
化学品 鱼类生殖毒性短期试验方法 Chemicals—Fish short term reproduction toxicity assay	GB/T 35517—2017	现行 In operation	2018-07-01
化学品 鱼类雌激素、雄激素和芳香酶抑制活性试验方法 Chemicals—Fish assay for oestrogenic and androgenic activity, and aromatase inhibition	GB/T 35515—2017	现行 In operation	2018-07-01
水质急性毒性的测定 斑马鱼卵法 Water quality—Determination of the acute toxicity—Zebrafish (*Danio rerio*) eggs method	待定 Pending	提交送审稿 Draft for review has been submitted	待定 Pending

在国内标准制定上,1992 年出台的《水质 物质对淡水鱼(斑马鱼)急性毒性测定方法》(GB/T 13267—91)中首次将斑马鱼用于水质检测,经过 20 余年的发展,已有 13 个国家标准将斑马鱼作为推荐物种用于测试水质、农药、废水和化学品的毒性(表 6)。2015 年 11 月原环境保护部发布《关于征求国家环境保护标准<城镇污水处理厂污染物排放标准>(征求意见稿)意见的函》(环办函〔2015〕1782 号),并发布《城镇污水处理厂污染物排放标准》征求意见稿和征求意见稿编制说明向社会征求意见,首次增加综合毒性指标来反映排放废水的综合毒性,包括发光细菌、大型溞、水藻和斑马鱼胚胎急性毒性。

3　展望(Outlook)

斑马鱼以其卓越的生物学特性成为毒理学研究中最重要的模式生物之一,是毒性测试和其他生物研究的哺乳动物模型的一种可替代方案,其全生命周期毒性检测技术已在重金属、有机污染物及内分泌干扰物质等方面有了较为广泛的研究,相关标准正在推进和完善。本文通过综述斑马鱼全生命周期毒性测试的研究进展,对未来开展相关研究提出以下几点建议:

(1)斑马鱼不同的生命阶段有不同的应用,能满足不同的需求,以特定的毒性终点来表征化合物的毒性。在胚胎阶段确定更合适的分子标记物作为毒性测试指标及建立短期毒性测试和长期毒性测试之间的关系,可初步判断有毒化合物的毒性类型及潜在危害程度,从而使斑马鱼胚胎模型更好地为环境管理决策服务。

(2)近年来基因组学等组学技术兴起,转基因斑马鱼进行环境污染物毒理学研究成为生态毒理学研究新热点。随着各种转基因斑马鱼的开发和利用,其灵敏度和效率也不断提高,利用斑马鱼的基因表达来研究毒性机制将更有利于以斑马鱼为模型研究环境污染物对人类健康的影响。

(3)目前国内尚没有一份针对某一化学品的斑马鱼整个生命周期和多阶段毒性试验的标准规范,建立一套斑马鱼整个生命周期和多阶段毒性试验的标准规范并对重金属、内分泌干扰物和有机污染物进行毒性评价具有较大的研究价值,对有毒化合物的生态风险评估以及水体污染的生物监测具有一定的指导意义,这将在以后的生态毒理学领域中具有较大的研究价值。

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